510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE

A. 510(k) Number:

K043215

B. Purpose for Submission:

New Device

C. Measurand:

Lymphocyte, CD4

D. Type of Test:

Quantitative, flow cytometry

E. Applicant:

Beckman Coulter, Inc.

F. Proprietary and Established Names:

FlowCARE™ PLG CD4 Reagent

G. Regulatory Information:

1. Regulation section:

   21 CFR 864.5220, Automated Differential Cell Counter

2. Classification:

   Class II

3. Product code:

   GKZ, Counter, Differential Cell

4. Panel:

   Hematology (81)

H. Intended Use:

1. Intended use(s):

   The FlowCARE PLG CD4 Reagent is for use on the Coulter EPICS XL / XL-MCL or equivalent flow cytometer. The reagent combines two fluorescent-labeled monoclonal antibodies in a single reagent formulation. It is intended "For In-Vitro Diagnostic Use" for the enumeration of CD4 absolute cell count and CD4 lymphocyte percentage in combination with a White Blood Cell(WBC) Count from a hematology instrument as a dual platform measurement, or independently when used in combination with Flow-Count Fluorospheres as a single platform measurement.

2. Indication(s) for use:

   The FlowCARE PLG CD4 Reagent is for use on the Coulter EPICS XL / XL-MCL or equivalent flow cytometer. The reagent combines two fluorescent-labeled monoclonal antibodies in a single reagent formulation. It is intended "For in-Vitro Diagnostic Use" for the enumeration of CD4 absolute cell count and CD4 lymphocyte percentage in combination with a White Blood Cell(WBC) Count from a hematology instrument as a dual platform measurement, or independently when used in combination with Flow-Count Fluorospheres as a single platform measurement.

   The FlowCARE PLG CD4 Reagent provides the ability to measure CD4+ cells by utilizing a pan-leucocyte gating (PLG) approach where CD4+ T-cell enumeration is based on the use of all leukocytes, instead of on lymphocytes only, as the matching common denominator between the hematology generated WBC and the flow cytometric enumeration. A sequential gating strategy is used to include all CD45+ leukocytes and to measure the CD4+ % of total leukocyte values generated from this gate.

3. Special conditions for use statement(s):

   Not applicable.

4. Special instrument requirements:

   The FlowCARE PLG CD4 Reagent is designed for use on the Coulter EPICS XL / XL-MCL or an equivalent flow cytometer with a 488 nm laser capable of detecting light scatter (forward and side) and a minimum of two-color fluorescence emission detectable in the following ranges: 515-545nm and 562-607nm.

I. Device Description:

FlowCARE PLG CD4 Reagent consists of a two-color antibody reagent composed of CD45-FITC and CD4-PE. The assay is performed on the EPICS XL, Cytomics FC 500, or equivalent Flow Cytometer using appropriate quality control reagents in combination with an optional absolute count reagent, Flow-Count Fluorospheres for determination CD4 absolute counts as a single platform measurement, or in combination with a White Blood Cell Count from a hematology analyzer as a dual platform measurement.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   tetraONE System for EPICS XL Flow Cytometry System with CYTO-STAT tetraCHROME CD45-FITC/CD4-RD1/CD8-ECD/CD3-PC5 Monoclonal Antibody Reagent

2. Predicate 510(k) number(s):

   K990172

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
	FlowCARE PLG CD4+ Reagent	***tetraONE System for EPICS XL Flow Cytometry System***
T-lymphocyte enumeration	CD4+	CD4+
Analytical instrumentation	EPICS XL-MCL flow cytometer or equivalent	EPICS XL-MCL flow cytometer
Analysis Reagent	Uses CD45 FITC and CD4-PE(RD1) monoclonal dye conjugates are identical to CD45-FITC/CD4-RD1 tetraCHROME reagent components	Uses CYTO-STAT tetraCHROME CD45-FITC/CD4-RD-1/CD8-ECD/CD3PC5
Analysis Reagent	Flow-Count Fluorospheres absolute count reagent or equivalent	Flow-Count Fluorospheres absolute count reagent
Setup Reagent	- Flow-Set	Same

Similarities		
Item	Device	Predicate
	Fluorospheres - CYTO-COMP Cell Kit - CYTO-COMP Reagent Kit	
QC Reagent	- IMMUNO-TROL Control Cells - IMMUNO-TROL Low Control Cells	Same

Differences		
Item	Device	Predicate
Analysis Reagents	2-color fluorochrome reagent	4-color fluorochrome reagent
Analysis Software	Manual analysis using customer created protocols according to package insert	System II Automated analysis using cellSTAT 3D algorithm
Flow Cytometer	EPICS XL-MCL, Cytomics FC500,or any equivalent flow cytometry system	EPIC XL-MCL system only
Specimen Age	≤ 120 hours (5 days)* *The specimen age limit for dual platform measurement is dependent upon the claim for the hematology analyzer but not to exceed five days.	- ≤ 6 hours (with automated software - ≤ 72 hours (without automated software, tetraCHROME CD45-FITC/CD4-RD/CD8-ECD/CD3-PC5)
Intended Use	Enumeration of CD4+ T-Lymphocytes only	- Enumeration of total T, B and NK lymphocytes - Enumeration of three major T-lymphocyte subset populations (CD3,CD4, CD8)

K. Standard/Guidance Document Referenced (if applicable):

Class II Special Controls Guidance Document: Premarket Notifications for Automated Differential Cell Counters for Immature or Abnormal Blood Cells; Final Guidance for Industry and FDA.

EP9-A2, *Method Comparison and Bias Estimation Using Patient Samples,* Approved Standard-Second Edition, NCCLS.

EP5-A, *Evaluation of Precision Performance of Clinical Chemistry Devices,* Approved Guideline, NCCLS.

Guidelines for the evaluation of blood cell analyzers including those used for differential leucocyte and reticulocyte counting and cell marker applications. International Council for Standardization in Haematology: Prepared by the ICSH expert panel on cytometry: Clin. Lab Haematol, 16(2): 157-174, 1994.

H04-A4 *Procedures and Devices for the Collection of Diagnostic Blood Specimens by Skin Puncture*; Approved Standard-Fifth Edition, NCCLS

L. Test Principle:

This test depends on the ability of a monoclonal antibody to bind to the surface of cells expressing discrete antigenic determinants. Specific cell staining is accomplished by incubating whole blood with the monoclonal antibody reagent. The FlowCARE PLG CD4 Monoclonal Antibody Reagent is a combination of two murine monoclonal antibodies, each conjugated to a specific fluorochrome and specific for different cell surface antigens.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. Precision/Reproducibility:

   Within Run

   The percent positive and CD4 absolute count values were determined using two levels of control, run in duplicate, twice each day for up to 20 days at three geographically diverse sites using the FlowCARE PLG CD4 Monoclonal Antibody Reagent and analyzed on a Coulter EPICS XL/XL-MCL. Measurements (% positive and absolute counts) for CD4+

lymphocytes were within assay range as determined in the control product package inserts. Results are as follows:

% CD4+ Lymphocytes

		Control 1		Control 2	
	n	Mean %	SD	Mean %	SD
Site 1	80	48.0	0.6	21.9	0.8
Site 2	80	48.1	0.7	22.2	0.7
Site 3	68	48.2	0.9	22.0	0.6
Pooled Results	228	48.1	0.8	22.0	0.7

CD4+ Absolute Counts

		Control 1		Control 2	
	n	Mean	SD	Mean	SD
Site 1	80	627	24	129	7
Site 2	80	641	20	133	4
Site 3	68	664	16	137	6
Pooled Results	228	639	21	133	6

b. Linearity/assay reportable range:

Three replicate measurements were made at each of eight serial dilutions of a concentrated preparation of Control cells to achieve a range of CD4+ lymphocyte concentrations. Cell were stained with the FlowCARE PLG CD4 reagent and analyzed by flow cytometry Coulter (EPICS XL/XL-MCL). Regression analysis as expressed in absolute counts (within the range of 0-5000 cell/µl) is as follows:

$y = 1.0416x + 12.558 \quad R^2 = 0.9996 \quad R = 0.9998$

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

Unopened reagent is stable for the dating period shown on the label when stored at 2-8^{o} C. Opened vial is stable for 90 days when stored at 2-8^{o}C.

d. Detection limit:

Not Applicable

e. Analytical specificity:

Not Applicable

f. Assay cut-off:

Not Applicable

2. Comparison studies:

a. Method comparison with predicate device:

A comparison study between FlowCARE PLG CD4 Monoclonal Antibody Reagent and the predicate device (tetraCHROME CD45-FITC/CD4-RD1/CD8-ECD/CD3-PC5 Monoclonal Antibody Reagent) was performed on 132 normal and 165 abnormal whole blood samples by flow cytometry (Coulter EPICS XL/XL-MCL). Data was collected at three independent external sites and one internal site. Regression analysis is as follows:

% CD4+ Lymphocytes
$y = 0.969x + 0.1416$, $R^2 = 0.9937$, $R = 0.9968$

CD4 Absolute Counts (Dual Platform) (0-1800 cell/µl range)
$y = 1.0526x – 13.184$, $R^2 = 0.979$, $R = 0.9894$

CD4 Absolute Counts (Single Platform) (0-1800 cell/µl range)
$y = 1.0289x – 15.244$, $R^2 = 0.967$, $R = 0.9833$

b. Matrix comparison:

Not applicable

3. Clinical studies:

a. Clinical Sensitivity:

Not aplicable

b. Clinical specificity:

Not applicable

c. Other clinical supportive data (when a. and b. are not applicable):

Not applicable

4. Clinical cut-off:

Not applicable

5. Expected values/Reference range:

Whole blood samples were collected from 132 apparently healthy (geographically diverse) males and females unselected as to race or age. The samples were stained with FlowCARE PLG CD4 reagent and CD4+ cell values were determined by flow cytometry (Coulter EPICS XL/XL-MCL). The results are as follows.

Normal Whole Blood

	N	Min	Max	Mean	SD
%CD4 Lymphocytes	132	23.4	71.0	47.6	9.6
CD4 Absolute Count (Dual Platform)	132	296	1856	949	299
CD4 Absolute Count (Single Platform)	132	288	1861	918	304

These values are intended as representative values only. Each laboratory should establish its own expected values from the local population of normal donors.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.